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CYTOGENETIC CHANGES IN BONE MARROW CELLS OF WISTAR RATS INDUCED BY LEVAMISOLE HYDROCHLORIDE

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"Levamisol" is an antiparasitic drug produced by ICN-Galenika (Yugoslavia). This anthelmintic was cytogenetically analyzed in vivo in an experiment on three groups of Wistar rats. The control group of rats was treated with saline. Experimental animals were injected intraperitoneally with two therapeutic doses of the anthelmintic (2,2 mg levamisole hydrochloride/kg b. w. and 4,4 mg levamisole hydrochloride/kg b.w.) during a seven day treatment period. Cytogenetic changes of bone marrow cells were evaluated by determination of their mitotic index, as well as structural and numerical chromosomal aberrations. Both tested doses of the anthelmintic induced an increase of mitotic index, aneuploidy, polyploidy and structural chromosomal changes of the break and insertion type of lesion. Insertions were observed on the first pair of autosomes.

Key words: Levamisole Hydrochloride, Wistar Rats, Cytogenetic Changes, Bone Marrow Cells, insertion Is [q21–q22].

INTRODUCTION

Levamisole hydrochloride is an active principe of many anthelmintic drugs widely used in roundworm and hookworm infections. Also, it is used as an adjuvant and immunostimulant in malignant diseases or in the acquired immunodeficiency syndrome. When levamisole hydrochloride is used for long periods, adverse and side-effects in humans are frequent (Amery and Butterworth 1983). These side-effcts include skin rash, nausea, vomiting, agranulocytosis and others. Also, the application of levamisole for long periods causes side-effects on the liver, endocrine and nervous systems.

The potential genotoxicity of levamisole was studied in vivo and in vitro by many authors, e. g. Vazques-Escobosa and Gomez-Estrada (1981), Aleksić

(1993) etc. Thus, Vazques-Escobosa and Gomez-Estrada (1981) pointed to the absence of the clastogenic effect of levamisole. However, the genotoxicological characterization of levamisole in PHA-stimulated human lymphocytes by Aleksić (1993) showed that the drug statistically increased the sister-chromatid exchange frequencies (SCE) in concentrations higher that 15 micrograms per mililiter of medium.

In veterinary medicine levamisole is also used as an anthelmintic against intestinal nematode worms. FOA/WHO (1990) recommended maximum residue limits of levamisole in food of animal origin. For all species it is 10 micrograms per kg of edible, tissues and milk. These organizations require further toxicological investigations including an assessment of the incidence of adverse haematological effects due to levamisole or its major metabolites in domestic animals.

The aim of this study was to determine cytogenetic changes of bone marrow cells that occur in Wistar rats after treatment with levamisole in therapeutic doses.

MATERIALS AND METHODS

Cytogenetic effects of the commercial anthelmintic "Levamisol" (ICN-Galenika, Yugoslavia) were studied on bone marrow cells. The experiment was performed on 6 to 7-week old male Wistar rats (Body mass from 150 to 200 g). All 54 rats in the experiment were divided into three groups.

The control group (C) was treated with saline. The first experimental group (E1) of rats was injected with levamisole hydrochloride (2,2 mg/kg b.w.). The second experimental group (E_2) of animals received 4,4 mg levamisole hydrochloride/kg b. w.

Levamisole hydrochloride was applied intraperitoneally as seven injections every day. On the last day of the experimental treatment all rats were intraperitoneally injected with colchicine and sacrificed.

Mitotic figures of metaphase chromosomes were obtained after the preparation of bone marrow cells by the method of Hsu and Patton (1969) somewhat modified by Zimonjić et al. 1990. G-banding method was done by the method of Seabright et al. 1971, somewhat modified by Levan (1974) and Ronne (1991).

The cytogenetic effects of levamisole hydrochloride on bone marrow cells of Wistar rats were evaluated by observing several parameters i. e., the mitotic activity (mitotic index = MI), and the appearance of numerical and structural chromosomal aberrations.

RESULTS AND DISCUSSION

The values of the mitotic index, numerical and structural chromosomal changes for the control group and two treated groups of rats are shown in Tables 1,2 and 3.

Table 1. Mitotic activity of bone marrow cells in rats treated with different concentrations of levamisole hydrochloride

Group of rats and dose of levamisole hydrochloride	No. of cells observed per animal		Mitotic index (MI)
	No.	%	X ± SD
C*	50	100	6.00 ∓ 0.26
E_1 2.2 mg/kg b.w.	50	100	8.50 ∓ 0.62
E_2 4.4 mg/kg b.w.	50	100	11.93 ∓ 0.60

Legend:
C = Control group treated with saline
E_1 = The first experimental group of rats treated with 2,2 mg levamisole hydrochloride kg b.w.
E_2 = The second experimental group injected with levamisole hydrochloride in the dose of 4,4, mg/kg b. w.
SD = Standard deviation

The lowest value of the mitotic index was observed in the control group of rats treated only with saline (6,00 ± 0,26), while the highest level of mitotic activity of bone marrow cells was estimated in the second experimental group injected with levamisole hydrochloride at the dose of 4,4 mg/kg b. w. (11,93 ± 0.60). In the first experimental group of rats treated with levamisole hydrochloride at the dose of 2,2 mg/kg b. w. this value was 8,50 ± 0.62. From the results obtained it is clear that levamisole hydrochloride stimulated bone marrow cells to divide. The increasing values of the mitotic index point to a mitogenic effect of this anthelmintic. Our results particularly confirm the statements of Janssen (1976) and Renoux (1980). These authors pointed to the immunostimulative effect of levamisole in human patients but they also noted that stimulation above the normal level did not seem to occur. According to them levamisole influenced host defences by modulating the cell-mediated immune response and restored depressed T-cell function.

The numerical and structural chromosomal changes in Wistar rats induced by levamisole hydrochloride are recorded in Tables 2 and 3. It can be concluded that levamisole hydrochloride induced numerical chromosomal changes of the polyploid and aneuploid type and structural chromosomal abberrations of the lesion, break and insertion type in the treated rats.

Table 2. Numerical chromosomal changes induced by levamisole hydrochloride

Group of rats and dose of levamisole hydrochloride	No. of cells observed per animal		Numerical chromosomal changes			
			Aneuploid cells		Polyploid cells	
	No.	%	$\bar{X} \pm SD$	%	$\bar{X} \pm SD$	%
C	50	100	4.50 ± 1.07	0.75	0.00 ± 0.00	0.00
E_1 2.2 mg/kg b.w.	50	100	67.00 ± 3.31	11.17	10.50 ± 1.31	1.75
E_2 4.4 mg/kg b.w.	50	100	111.25 ± 7.70	18.54	10.38 ± 1.77	1.73

Legend:
C = Control group treated with saline
E_1 = The first experimental group of rats treated with 2,2 mg levamisole hydrochloride kg b.w.
E_2 = The second experimental group injected with levamisole hydrochloride in the dose of 4,4 mg/kg b.w.
SD = Standard deviation

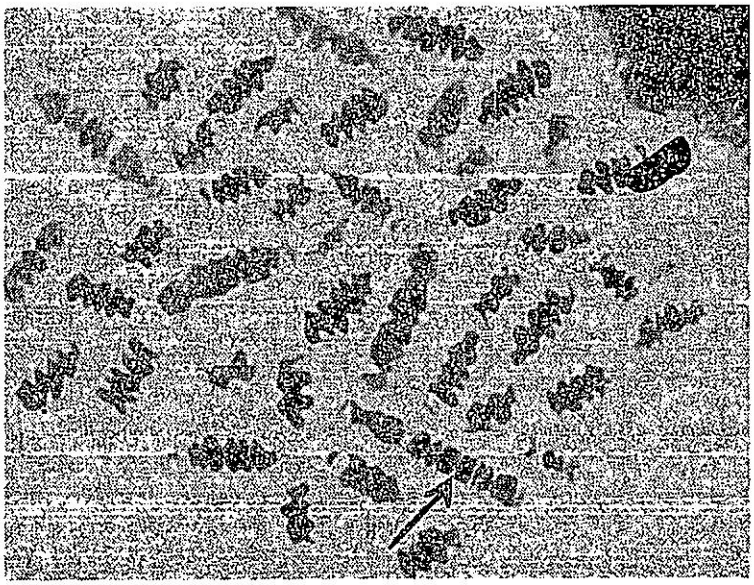

Figure 1. Aberrant karyotype of Wistar rat with insertion on the first autosome (The arrow points to the insertion marked as 1A band).

At the investigated doses levamisole induced both types of chromosomal aberrations, namely, numerical and structural changes. The number of aneuploid and polyploid cells was higher in both experimental groups of rats compared to the control group treated with saline. A certain number of aneuploid cells normally occurs as a consequence of for the method of chromosome preparation. The same situation occurred in the example of structural chromosomal changes

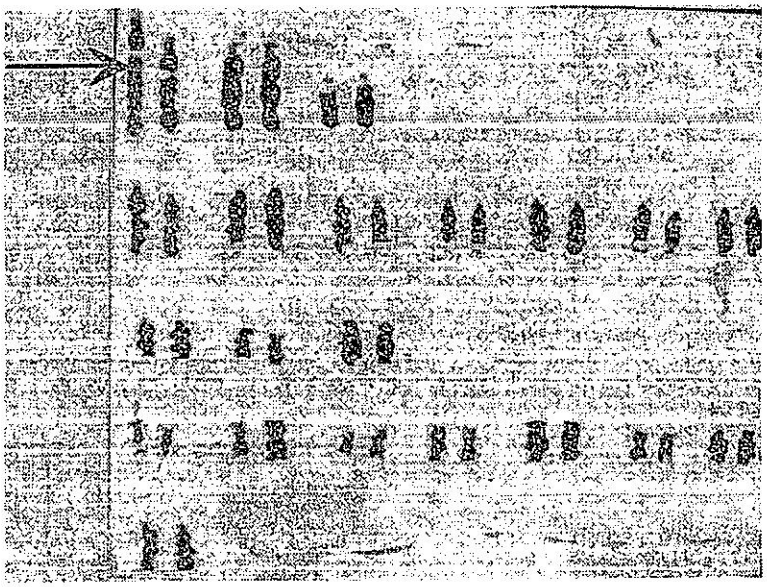

Figure 2. The first pair of autosomes with a chromosomal change of the insertion type. (The arrow points to the insertion marked as 1A band).

shown in Table 3. Both investigated doses of levamisole induced changes in chromosomal structure of the lesion, break and insertion type. Insertions were observed only on the first pair of autosomes (Figure 1,2 and 3). This chromosomal change was located between bands q21 and q22 (Figure 3). Therefore, we consider that therapeutic doses of levamisole hydrochloride administrated in the treatment period of seven days exhibited clastogenic properties. Similar results were obtained by other authors who investigated the genotoxicity of this antheimintic in vitro (e.g. Aleksić 1993 etc.) Our results did not support the statement of Vazques-Escobosa and Gomez-Estrada (1981) that levamisole could not induce chromosome fractures. Also, we consider that further investigations on the genotoxicity of levamisole could give an adequate explanation for the chromosomal changes of the insertion type on the first pair of autosomes in Wistar rats. Moreover, it is possible that this type of chromosomal change is not produced by levamisole but by its metabolites formed in the rats during the detoxycation proccess (Stanimirović 1995). Similar results have been obtained in our previous experiments on rabbits and mice (Stanimirović et al., 1993, Stanimirović et al. 1995). Also, we estimated structural chromosomal changes of the insertion type on autosomes of these species. We concluded that this type of chromosomal aberration was the consequence of clastogenic effects of some adverse maters in food or in the environment such as pesticides, food additives etc. According to Traut et al. (1996), changes of chromosomal macromorphology of similar types could be the consequence of repetition of endogenous sequences by the consequence of repetition of endogenous sequences by duplication and amplification.

Table 3. Structural chromosomal changes induced by levamisole hydrochloride

Group of rats and dose of levamisole hydrochloride	No. of cells observed per animal		Structural chromosomal changes					
			Lesions		Breaks		Insertions	
	No.	%	$\bar{X} \pm SD$	%	$\bar{X} \pm SD$	%	$\bar{X} \pm SD$	%
C	50	100	3.25 + 0.71	0.54	1.38 + 0.75	0.23	0.00 + 0.00	0.00
E_1 2.2 mg/kg b.w.	50	100	5.50 + 0.93	0.92	10.63 + 1.77	1.77	6.14 + 0.78	13.23
E_2 4.4 mg/kg b.w.	50	100	6.37 + 1.06	1.06	6.50 + 0.76	1.08	12.57 + 1.07	27.00

Legend:
C = Control group treated with saline
E_1 = The first experimental group of rats treated with 2,2 mg levamisole hydrochloride/kg b.w.
E_2 = The second experimental group injected with levamisole hydrochloride in the dose of 4,4 mg/kg b.w.
SD = Standard deviation

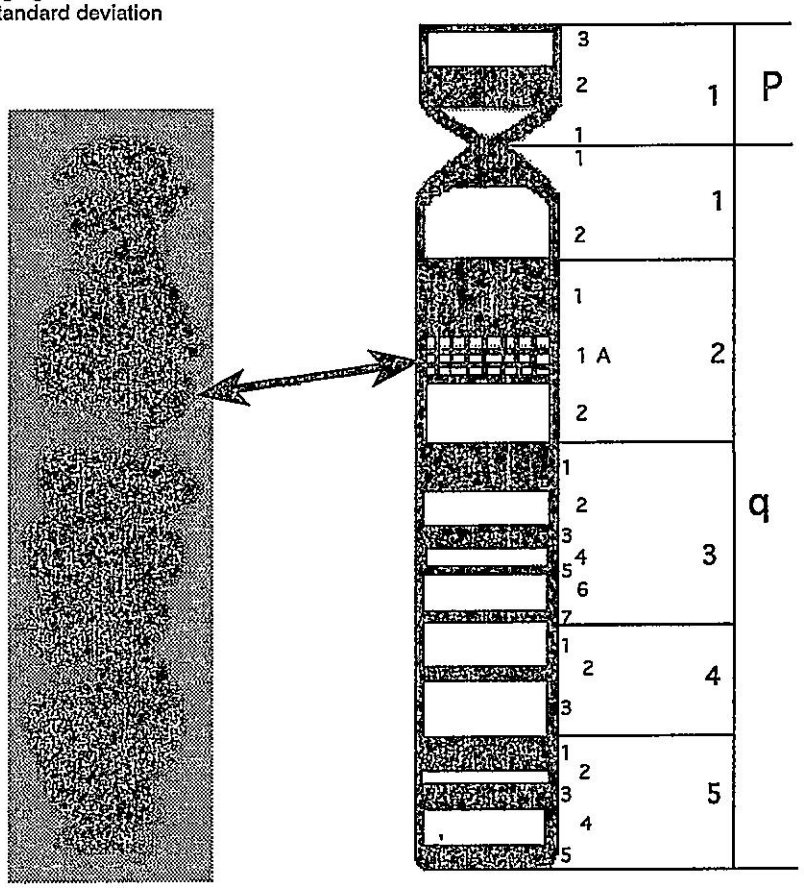

Figure 3. Idiogram of the first pair of autosomes with the insertion market as 1A band).

REFERENCES

1. *Aleksić, N.* 1993. Genotoksikološka in vitro karakterizacija nekih savremenih antiparacitika. Doktorska disertacija. Veterinarski fakultet, Beograd.
2. *Amery, V. K., and Butterworth, B. S.* 1983. Reiwiey commentary: the dosage regimen of levamisole in cancer is related to efficiency and safety. Int. J. Immunopharmacol. 5, 1-9.
3. *FAO/WHO* 1990. Evaluation of certain veterinary drug residues in food: Thirty-sixth report of the joint FAO/WHO expert committee on food additives. WHO Tech. Rep. Ser.799.
4. *Hisu T. C. and Patton, J. I.* 1969. Bone marrow preparations for chromosome studies. In: Benirischke V. (ed.). Comparative Mammalian cytogenetics. Springer Verlag, Berlin, Heidelberg, New York, 454-460.
5. *Janssen, P. A. J.* 1976. The levamisole story. Prog. Drug. Res. 20, 347-383.
6. *Levan, G.* 1974. Nomenclature for G-bands in rat chromosomes. Hereditas 77, 37-52.
7. *Renoux G.* 1980. The general immunopharmacology of levamisole. Drugs 20, 89-99.
8. *Ronne, M.* 1991. High-resolution banding: present aspects. Genet. Sel. Evol. 23, suppl. 1, 49s-55s.
9. *Seabright, M.* 1971. A rapid banding technique for human chromosomes. Lancet II, 971.
10. *Stanimirović, Z.* 1995. Komparativne analize prirodnog i indukovanogpolimorfizma hromozoma kod vrste us musculus, Linne 1758. Doktorska disertacija Biološki fakultet Beograd.
11. *Staniirović, Z. Soldatović, B., Dimitrijević, Sandra, Vučinić, Mmarijana and Djokić, D.* 1993. Aberrant karyotype of an experimental rabbit (Oryctologus cuniculus, Linnaeus, 1758) with a heterozygous first autosomal pair. Acta Veterinaria, 55-60.
12. *Stanimirović, Z., Vučinić, Marijana, Soldatović, B and Vučićević, Marijana* 1995. A large acrocentric chromosome in the first pair of autosomes in natural populations of Mus musculus, Linne 1758. Acta Veterinaria, 155-160.
13. *Traut, W., Weichenhan, D., Kunze, B. and Winking, H.* 1996. Long-range repeat families as a component of the mammalian genome. In: P. E. Brandham and M. D. Bennett (editors) Kew Chromosome Conference IV, pp. 95-107, Royal Botanic Gardens, Kew.
14. *Vazques-Escobosa, C. and Gomez-Estrada,* 1981. Levamisole does not induce chroosome fractures. Arch. Invest. Med. 12, 173-178.
15. *Zimonjić, D. B. Savković, N. and Anđelković, M.* 1990. Genotoksični agensi - efekti, principi i metodologija detekcije. Naučna knjiga, Beograd.

CITOGENETIČKE PROMENE ĆELIJA KOSTNE SRŽI PACOVA WISTAR SOJA INDUKOVANE LEVAMIZOL HIDROHLORIDOM

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SADRŽAJ

U radu su ispitivani citogenetički efekti komercijalnog preparata "Levamizola" (ICN Galenika, Jugoslavija) koji se u veterinarskoj medicini primenjuje kao antihelintik. Ovo sredstvo je intraperitonealno u toku sedmodnevnog tretmana aplikovano pacovima Wistar soja. Ogled je izveden na mužjacima starim

sedam nedelja, telesne mase 150 do 200 g. Sve životinje su bile podeljene u tri grupe:

C - kontrolna grupa koja je u toku sedam dana tretirana fiziološkim rastvorom,

E_1- prva eksperimentalna grupa, koja je u toku sedmodnevnog dobijala terapijsku dozu levamizol hidrohlorida od 2,2 mg na kilogram telesne mase.

E_2- druga eksperimentalna grupa, koja jeu toku sedmodnevnog tretmana dobijala terapijsku dozu levamizol hidrohlorida od 4,4 mg/kg telesne mase.

Citogenetičkom pretragom metafaznih figura ćelija kostne srži žrtvovanih životinja ustanovljeno je da obe terapeutske doze levamizol hidrohlorida ispoljavaju mitogene efekte stimulišući ćelije kostne srži na deobu, kao i da poseduju sposobnost indukcije numeričkih i strukturnih hromozomskih aberacija u tipu aneuploidija, poliploidija, lezija, prekida i insercija. Prisustvo insercija ustanovljeno je na q kraku prvog para autozoma između traka q21 i q22, gde je bila inserirana traka q21A nastala endogenom duplikacijom ili amplifikacijom DNK regiona.